

# PATENT SPECIFICATION

NO DRAWINGS

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## COMPLETE SPECIFICATION

### Encapsulated Emulsions and Processes for their Preparation

We, THE UPJOHN COMPANY, a corporation organised and existing under the laws of the State of Delaware, United States of America, of 301, Henrietta Street, Kalamazoo, State of Michigan, United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to a process of encapsulation by liquid-liquid phase separation and to products resulting therefrom, and more particularly to a process of coacervation for encapsulating particles consisting of an oil-in-hydrophilic liquid emulsion and to the products thereof.

As employed herein, the term lipophilic is applied to those surfaces having stronger attractive forces for low dielectric constant and non-polar media than for high dielectric constant and polar media. The term hydrophilic refers to those surfaces having stronger attractive forces for high dielectric constant and polar media than for low dielectric constant and non-polar media.

According to the novel process of this invention, the novel products hereof are prepared by first forming a primary oil-in-hydrophilic liquid emulsion (the oil being a lipophilic liquid) containing one or more thickening agents as hereinafter defined in the hydrophilic liquid phase. The primary emulsion is then dispersed in a coacervating solution comprising a coacervating agent and an aqueous sol or solution of a coacervating colloid at a temperature above the gel point of the said coacervating colloid to produce a secondary emulsion, whereupon a coacervate deposits about the particles of the said secondary emulsion, the particles being composed of the primary emulsion.

Liquid-liquid phase separation refers to the separation of a solution or a sol of a poly-

mer or combination of polymers or colloid into two distinct liquid phases, one designated as the polymer-rich phase and the other the polymer-poor phase. Where the polymer-rich and polymer-poor phases are colloidal sols rather than true solutions, the phenomenon of phase separation is herein designated as coacervation. Thus, a coacervate is a polymer-rich sol which has separated from an original single-phase polymeric dispersion (either a solution or a sol), leaving behind a polymer-poor sol or equilibrium liquid. The coacervate appears initially as a fine dispersion of microscopic droplets of polymer in the equilibrium liquid. When formed in a pure colloidal system, these droplets are essentially homogeneously dispersed. However, if foreign materials are present in the original dispersion, the coacervate tends to form around these materials. Technically, the term "coacervation" therefore relates to the process by which the liquid colloidal concentrate or coacervate is formed as a phase entity of the initial sol or solution. In its practical aspect, and as employed herein, "coacervation" relates to the process by which "foreign" materials present in the sol when the coacervate is formed are enveloped or encapsulated by the coacervate. Where the coacervate consists of a single colloid, as herein, the process is termed "simple coacervation"; where more than one colloid is present in the coacervate, the process is called "complex coacervation".

Coacervation has long been known as a phenomenon primarily of academic interest, and only in recent years has it been developed in certain limited aspects for commercial utilization. However, even with this renewed interest in the subject, the technique has been successfully described only for the coating of oil droplets per se and of oil droplets containing dissolved or dispersed materials. British Patent Specification 751,600 discloses methods for encapsulating oil droplets by co-

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acervate coatings of the complex and simple types. Although the said patent describes the formation of coacervates from an oil-in-water emulsion, only the oil phase is actually encapsulated by the coacervate. Prior to the present invention, the encapsulation of an intact emulsion of any type by a coacervate had not been reported, and the important advantages of a coacervate-coated emulsion in which the phases thereof contain dissolved or suspended active ingredients as hereinafter specified were not heretofore available. In addition, no method was known whereby a coacervate could be deposited on any surface, emulsion or otherwise, displaying hydrophilic qualities or being nominally hydrophilic in character, as, for example, an aqueous or a water-soluble surface.

It has now been unexpectedly found, however, that a hydrophilic surface, as for example, an aqueous surface, presented by the external phase of an emulsion, can be adapted to receive a coacervate membrane by providing at least one thickening agent, as herein defined, in the hydrophilic liquid or aqueous external phase of the said emulsion. The presence of the thickening agent, in necessary conjunction with the oil comprising the internal phase, in some unknown manner imparts to the outer surface of the external phase the surface characteristics required for coacervate deposition by rendering it lipophilic, or, at least, less hydrophilic than water.

The term "hydrophilic liquid" is herein intended to refer to water, aqueous solutions or suspensions, and non-aqueous solutions or suspensions immiscible in the oil phase of the primary emulsion. As used herein the term "active ingredient" refers to material which may be included in either or both phases of the primary emulsion and which does not substantially affect either the emulsification or coacervation process.

In addition to emulsions containing soluble or suspendable active ingredients in the hydrophilic liquid phase, the coacervates herein, by practice of the present invention, can be deposited about any oil-in-hydrophilic liquid emulsion containing dissolved or suspended active ingredients in the internal oil phase. The active ingredients to be dissolved or suspended in either the hydrophilic liquid or the oil phases are limited in selection only by the solubility, suspending characteristics or compatibility of the ingredients in both phases.

The present process and products resulting therefrom afford a new approach to the provision of impermeable coatings of high strength or coatings which permit a gradual release of contents for water-soluble materials broadly, a problem which has heretofore resisted solution by the known techniques of coacervation. For example, encapsulated emulsion particles can be prepared containing appropriate active ingredients in the emulsion phases for use as

sustained release fertilizers, plant growth hormone preparations and pesticides such as fungicides, nematocides, bactericides, viricides and the like for agricultural use. In addition, active ingredients can be incorporated in premixed foods which could not normally be included because of the loss on drying, the encapsulated ingredients being liberated by the shearing force exerted in a mixing step prior to actual use. Similarly, vitamins, notably combinations of water-soluble and oil-soluble vitamins, can be incorporated into dry cereal preparations for release in the body. Cosmetics can be prepared in which the topical agent is enclosed by impermeable but readily destructible coacervate shells. Pharmaceutical materials can be encapsulated for sustained release or delayed release in the body upon contact with a predetermined pH environment or enzyme system, or where stability, odour, taste or incompatibility problems are present. Such materials can be enclosed in coatings suitable for oral, topical or injectable use by regulation of the particle size and coating thickness, permeability and hardness, or by selection of coating components. Insecticides with selective toxicity for insects but which are relatively non-toxic toward humans can be encapsulated, for example, with coacervate coatings which are highly impermeable except in the presence of enzymes of the insects. Rodenticides which are effective on ingestion by the animals but which have odours that forewarn or repel them can likewise be coated by the method of this invention with virtually complete impermeability with respect to the odour.

As employed herein, the term "primary emulsion" is intended to refer to the oil-in-hydrophilic liquid emulsion initially formed from the hydrophilic liquid, with or without dissolved or suspended active ingredients, and, as the internal phase, the selected oil, with or without dissolved or suspended active ingredients. The selection of the oil is not critical and is dependent on the function to be served by the oil, i.e., as a solvent or suspending medium or as the external phase of the emulsion. Thus, virtually any animal, vegetable, mineral or synthetic oil can be employed for this purpose. Lanolin, corn oil, soybean oil, castor oil, cod liver oil and mineral oil are examples of such oils. The conventional emulsifying agents, such as esters of polyhydric alcohols, sorbitan derivatives and polyoxyethylene derivatives, are usually employed in preparing the emulsion. Selection of the particular surface active agent or combination of agents useful in preparing any particular emulsion can advantageously be made by reference to the HLB (hydrophilelipophile balance) system, as described in Remington's Practice of Pharmacy, 11th edition, Mack Publishing Company, 1956, page 191. As with all emulsion formation

problems, selection of the most suitable agents must ultimately be based on trial. Accordingly, a sample of the final emulsion should be checked, for example, by diluting and agitating with a relatively large volume of water, to determine that a stable emulsion of the type desired has actually been obtained. If a stable emulsion has been obtained, an emulsion is formed on dilution, but if the emulsion obtained was unstable, then on dilution the oil particles rise to the surface.

The term "thickening agent" as used herein refers to materials which are substantially insoluble in the oil phase of the primary emulsion and which will cause the external phase of the primary emulsion to have a surface which is lipophilic, or, at least, less hydrophilic than water. This permits a coacervate to deposit thereon. The presence of one or more thickening agents in the external phase of the primary emulsion is an essential element of this invention. To be operative in facilitating coacervation, however, these thickening agents require the presence of oil as internal phase, there being an as yet undetermined relationship or interdependence between the thickening agents and the oils. It is also frequently advantageous to include a thickening agent in the coacervating solution, as hereinafter defined. Suitable materials constituting the thickening agents hereof embrace the known natural and synthetic thickening agents (including derivatives of both), specifically including those alluded to in "Thickening Agents Used In Pharmacy", by Charles H. Becker, American Professional Pharmacist 20: 939 (October) 1954, as acacia, tragacanth, methyl cellulose, carboxymethylcellulose, and magnesium aluminum silicate, as well as other thickening agents such as the polyglycols, glycerin, and syrups. The specific amounts of these materials may vary with the particular agent and system involved and can be readily determined by routine experimentation. A range of from about 1 to about 20% by weight, based on the volume of the hydrophilic liquid phase, broadly represents a practicable range, with from about 5 to about 10% being preferred in most instances.

The term "coacervating colloid" is herein intended to refer to a gelable hydrophilic colloid in which, in the process of the invention, is employed in the form of an aqueous sol, or solution, which, on the addition of a "coacervating agent", forms a liquid colloid-rich and a colloid-poor phase, the colloid-rich phase depositing about single or aggregated emulsion particles of the primary emulsion dispersed in the "coacervating solution", the colloid-poor phase constituting the equilibrium liquid. Suitable gelable hydrophilic colloids include gelatin, agar-agar, albumen, alginates, casein, pectins, starch, and fibrinogen, the preferred colloid being gelatin. The ultimate thick-

ness of the coacervate enclosing the secondary emulsion particles will depend on the amount of colloid available for formation of the coacervate and the surface area of secondary emulsion particles to be encapsulated.

The term "coacervating agent" herein refers to materials capable of initiating the separation of a colloid-rich phase and a colloid-poor phase from an original single phase colloidal sol (or solution). Such substances contemplated by the term coacervating agent include (1) aqueous solutions of electrolytes, including organic and inorganic salts, e.g., salts having alkali or alkali-metal cations such as sodium, ammonium, magnesium, and potassium, and organic or inorganic anions such as sulphate, phosphate, acetate, and formate, and (2) liquids which are water-soluble and in which the coacervating colloid is less soluble than in water. A critical concentration of each coacervating agent exists below which coacervation will not occur. This concentration must be determined for each combination of coacervating colloids and coacervating agent by routine testing.

The term "coacervating solution" as used herein applies to the mixture of the coacervating agent and the sol or solution of the coacervating colloid, both as defined above, with or without a thickening agent, prior to the separation of the aforesaid colloid-rich phase (coacervate).

The term "secondary emulsion" refers to the emulsion formed when the primary emulsion is added to the coacervating solution before coacervation takes place. The secondary emulsion comprises the primary emulsion dispersed in the coacervating solution and exists as an entity of the mixture only until a coacervate is formed about the particles of the secondary emulsion.

In the preferred embodiment of this invention, a primary oil-in-water emulsion is prepared by emulsifying (1) a vegetable oil such as corn oil into (2) approximately an equal volume of an aqueous solution containing the desired active ingredient, together with a small quantity of methyl cellulose as a thickening agent. The preparation of the emulsion is carried out at approximately 55° C. A gelatin sol and the primary emulsion are introduced as a single stream into a sodium sulphate solution (coacervating agent) containing acacia (thickening agent) under constant and vigorous stirring to produce the secondary emulsion, the temperature being maintained at approximately 55° C. throughout the period of mixing. Coacervation occurs rapidly on contact of the gelatin and the primary emulsion with the sodium sulphate, the secondary emulsion existing only momentarily in the coacervating solution. The temperature of the equilibrium liquid containing the coacervate is then reduced to 5° C. over a period of about 30 minutes to gel the coacervate. After adjust-

ing the pH to the alkaline side, formaldehyde is added to the equilibrium liquid containing the now gelled coacervate to harden the coacervate, and the hardened coacervate is then filtered from the equilibrium liquid, washed and dried to give small particles of encapsulated emulsion.

In the preparation of the primary emulsion, the conventional emulsifying agents are normally employed, as previously indicated, to facilitate the establishment of and contribute to the stability of the primary emulsion, as well as to assure that the correct type of emulsion, i.e., oil-in-hydrophilic liquid, is obtained. Since the size of the final encapsulated emulsion particles depends in part on the size of the emulsion droplets of the primary emulsion, the degree of dispersion of the oil in the hydrophilic liquid should be regulated in accord with the desired particle size of the ultimately obtained coacervate.

The temperature at which the primary emulsion is prepared is of little consequence with respect to the functioning of the present process. However, it is necessary that the temperature at which coacervation is carried out be above the gel point of the coacervating colloid and within or closely approaching the gelling or thickening range of the thickening agent present in the hydrophilic liquid phase of the primary emulsion. Where methyl cellulose is employed as the thickening agent, the temperature of the coacervating solution should be about 50° C. provided that this temperature is above the gel point of the coacervating colloid. After the coacervate shell has enveloped the emulsion particles, the temperature is lowered below the gel point of the coacervating colloid. Where gelatin is employed as this component, reduction in the temperature to 30° C. or lower, depending on the type of gelatin used, preferably to about 5° C., will produce the desired gelation.

As indicated previously, the secondary emulsion exists during the interval between the first contact of all ingredients of the coacervating solution and the actual formation of the coacervate. The secondary emulsion consists of particles of the primary emulsion dispersed in the coacervating solution. If the primary emulsion and the solution of the electrolyte are added to the aqueous solution of the coacervating colloid, the secondary emulsion will persist until the concentration of the coacervating agent reaches the necessary level at which coacervation will occur. Where, for example, sodium sulphate solution is employed as the electrolyte, the critical concentration with gelatin as the coacervating colloid has been found to be approximately 7%. However, where, as by the preferred sequence, the coacervating colloid and the primary emulsion are added together to the electrolyte, a sufficient concentration of the

coacervating agent is present at all times during the said addition, and accordingly the secondary emulsion persists for only a short interval before coacervation takes place. Where the coacervating agent is a solvent in which the colloid is less soluble than it is in water, the solvent is added slowly to a mixture of the primary emulsion and the coacervating colloid with constant stirring to form the secondary emulsion. When the critical concentration range is reached for the particular colloid and solvent involved, coacervation will occur. Throughout either of the above procedures, the temperature must be above the gel point of the coacervating colloid.

The ultimate particle size of the coacervate product is dependent in part, as heretofore indicated, on the degree of dispersion or size of the oil particles of the primary emulsion. In addition, the particle size is of course a function of the thickness of the coacervate coating. Also of importance in this regard is the degree of dispersion of the primary emulsion and coacervating colloid in the coacervating solution. The more complete and rapid the mixing, the smaller are the secondary emulsion droplets that are presented as nuclei about which the coacervate will form, and hence the smaller will be the final coacervate units.

The gelling step is significant with respect to the permeability of the coacervate membrane. With many coacervate systems, instantaneous gelling of the warm coacervate, as by adding the warm coacervate to ice water, produces a coacervate membrane having high permeability. A prolonged period of slow cooling also favours a membrane of high permeability. With many coacervate systems the lowest permeability (or highest impermeability) is obtained with intermediate cooling rates. Thus, a highly impermeable coacervate coating is produced in the case of a gelatin coacervate on cooling the newly-formed coacervate to about 5° C. in a period of approximately 30 minutes with continuous stirring.

Following gelation of the liquid coacervate, the gelled coacervate optionally can be hardened, plasticized or otherwise treated to adapt it to the intended use. Treating the gelled coacervate for about 1 hour, with, for example, a 37% aqueous solution of formaldehyde under alkaline conditions produces a hardened coacervate shell which can then be dried.

Variations in the hardness of the coacervate shell can be obtained by varying the quantity of hardening agent and/or the period of contact therewith. Hardening likewise has considerable influence on the permeability of the coacervate, both with respect to the invasion of environmental fluids which would cause disintegration of the coating and to the containment of active ingredients which would

otherwise impart undesirable odour or taste characteristics to the product.

The finally treated coacervate can be separated by centrifuging, filtering, decanting or the like. This can be followed by drying by known methods, as by spray drying, freeze drying, air drying, or direct heating, optionally preceded by a washing step, to obtain a product essentially free of surface moisture. Such a product can then be formulated as a dry material.

A convenient and informative test for the integrity of a coacervate coating produced by the method of the present invention involves the incorporation of a soluble dye in the hydrophilic liquid phase of the primary emulsion. The coacervate is formed in the manner described and the resulting material, after gelling and, optionally, after hardening, is dispersed or immersed in the test liquid. The liquid is gently stirred to thoroughly expose all coacervate surfaces. Any dye escaping from the hydrophilic liquid phase through the coacervate shell is readily detectable in the test liquid.

The following examples are illustrative of the process and products of the present invention but are not to be construed as limiting the scope of the invention.

#### EXAMPLE 1.

An oil-in-water emulsion is prepared by emulsifying at 50° C., 50 ml. of corn oil into 50 ml. of water containing 2.5 gm. of methyl cellulose. A gelatin sol comprising 25 gm. of gelatin and 250 ml. of water is heated to 50° C., mixed with the emulsion, and added slowly to 250 ml. of a 20% sodium sulphate solution, also heated to 50° C. The sodium sulphate solution is vigorously stirred throughout the period of addition. The temperature of the mixture is lowered to 5° C. to gel the coacervate. Sufficient 10% sodium hydroxide solution is added to bring the pH to 9.5, followed by hardening of the coacervate with 25 ml. of 37% formaldehyde solution for 1 hour. The hardened coacervate is then filtered, washed and air dried at 80° C.

#### EXAMPLE 2.

Following the procedure of Example 1 but substituting the same quantities of peanut oil for the corn oil, tragacanth for the methyl cellulose, potassium chloride for the sodium sulphate and fibrinogen for the gelatin, there is produced a coacervate having substantially the same properties as the coacervate produced therein.

#### EXAMPLE 3.

An oil-in-water emulsion is prepared by emulsifying, at 50° C., 33 ml. of mineral oil into 25 ml. of water containing 2 gm. of magnesium aluminum silicate and 0.5 gm. alizarin cyanide green. A gelatin sol is prepared at 50° C from 12.5 gm. of gelatin and

125 ml. of water and is thoroughly mixed with the emulsion. The remaining mixture is added slowly to 125 ml. of a 20% sodium sulphate solution containing 37 gm. of acacia, the sodium sulphate solution being vigorously agitated throughout the period of addition to facilitate coacervate formation. The temperature of the equilibrium liquid containing the coacervate-coated emulsion is lowered to 5° C. and 10% sodium hydroxide is added to give a pH of 9.5. Thereafter, 12.5 ml. of 37% formaldehyde solution is added to harden the coacervate shell. After 5 hours, the resulting product is filtered from the mixture, washed and spray dried at 80° C. (exhaust temperature).

Exposure of the above coacervate to acid and alkaline test solutions indicates that a highly impermeable coating has been obtained.

#### EXAMPLE 4.

Following the procedure of Example 3 but substituting lanolin for the mineral oil as the oil phase of the oil-in-water emulsion, carboxymethylcellulose for the magnesium aluminum silicate as the thickening agent for the water phase of the primary emulsion, ammonium phosphate for the sodium sulphate as the coacervating agent, agar-agar for the gelatin as the coacervating colloid, and tragacanth for the acacia as the thickening agent for the coacervating solution is productive of a coacervate having properties similar to those obtained therein.

#### EXAMPLE 5.

An oil-in-ethyl alcohol emulsion is prepared by emulsifying, at 50° C., 33 ml. of peanut oil into 25 ml. of ethyl alcohol containing 2 gm. of methyl cellulose. A fibrinogen sol is prepared at 50° C. from 12.5 gm. of fibrinogen and 125 ml. of water and is thoroughly mixed with the emulsion. The resulting mixture is added slowly to 125 ml. of a 20% sodium sulphate solution containing 37 gm. of acacia, the sodium sulfate solution being vigorously agitated throughout the period of addition to facilitate the formation of the coacervate. The temperature is lowered to 5° C. to gel the coacervate and 10% sodium hydroxide is added to bring the pH to 9.5. Thereafter, 12.5 ml. of 37% formaldehyde solution is added to harden the coacervate shell. After standing for 5 hours, the resulting product is filtered from the mixture, washed and freeze dried at 40° C. and 0.01 mm. mercury vacuum.

The procedures of Examples 1 through 5 are likewise operable with other thickening agents for the hydrophilic liquid as previously described which impart to the external phase a quality which enables a coacervate to deposit thereon. Specifically, for example, equivalent amounts of acacia, tragacanth, carboxymethylcellulose, magnesium aluminum silicate,

the polyglycols, glycerin, and syrups can be employed.

WHAT WE CLAIM IS:—

1. A process for coating particles of an oil in hydrophilic liquid emulsion which comprises forming a primary oil-in-hydrophilic liquid emulsion containing at least one thickening agent in the hydrophilic liquid phase and dispersing the said primary emulsion in a coacervating solution comprising a coacervating agent and an aqueous sol or solution of a coacervating colloid as hereinbefore defined at a temperature above the gel point of the coacervating colloid to form a secondary emulsion whereby a coacervate is deposited about the particles composed of the primary emulsion.
2. A process as claimed in Claim 1 wherein the hydrophilic liquid and/or the oil used in forming the primary oil-in-hydrophilic liquid emulsion contains dissolved or suspended active ingredients as hereinbefore specified.
3. A process as claimed in Claim 1 or 2 wherein the primary oil-in-hydrophilic liquid emulsion is formed by emulsifying an animal, vegetable, mineral or synthetic oil and the hydrophilic liquid in the presence of an emulsifying agent.
4. A process as claimed in any preceding claim in which the coacervating solution contains a thickening agent.
5. A process as claimed in Claim 4, in which the thickening agent is methyl cellulose, carboxymethyl cellulose, acacia, tragacanth or magnesium aluminium silicate.
6. A process as claimed in any preceding claim in which the hydrophilic liquid of the primary emulsion is water.
7. A process as claimed in any preceding claim in which the coacervating colloid is gelatin, fibrinogen or agar-agar.
8. A process as claimed in any preceding claim in which the medium containing the coacervate coated particles is cooled to gel the coacervating colloid.
9. A process as claimed in Claim 8 wherein the coated particles are separated by centrifuging, filtering or decanting and then sub-

jected to spray drying, freeze drying, air drying or direct heating.

10. A process for coating particles of a primary oil-in-water emulsion which comprises preparing a primary oil-in-water emulsion by emulsifying a vegetable oil into about an equal volume of an aqueous solution containing an active ingredient as hereinbefore specified with methyl cellulose, introducing a gelatin sol and the primary emulsion in a single stream into an agitated sodium sulphate solution containing acacia at a temperature maintained at about 55° centigrade throughout the period of mixing, reducing the temperature to 5° centigrade over a period of about 30 minutes to gel the coacervate, adjusting the pH to alkaline and adding formaldehyde to the equilibrium liquid containing the now gelled coacervate to harden the latter, and then filtering the coacervate from the equilibrium liquid and washing and drying to give small particles of encapsulated emulsion.

11. A process as claimed in Claim 12 wherein the vegetable oil used is corn oil and the formation of the primary emulsion is carried out at about 55° centigrade in the presence of an emulsifying agent.

12. A capsule comprising an oil-in-hydrophilic liquid emulsion enclosed in a simple coacervate coating, the coacervating component of which is a gelable hydrophilic colloid.

13. A capsule as claimed in Claim 12 in which the hydrophilic liquid is water.

14. A capsule as claimed in Claim 12 or 13 in which the oil-in-hydrophilic liquid emulsion contains an active ingredient as hereinbefore specified dissolved or suspended therein.

15. A process for the preparation of an encapsulated oil-in-hydrophilic liquid emulsion substantially as herein described with reference to any of the examples.

16. An encapsulated oil-in-hydrophilic liquid emulsion when prepared by a process as claimed in any of Claims 1 to 11 or 15.

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